Diagnosing canine idiopathic hypereosinophilic syndrome

Otilia R. CRISTEA, Teodoru SOARE, Ana Maria GOANȚĂ, Lucian IONIȚĂ
Faculty of Veterinary Medicine Bucharest, USAMVB, Splaiul Independenței 105;
otilia.cristea@standardvet.ro; laboratorhistovet@gmail.com
ana_mv@yahoo.com; ionital@yahoo.com

Abstract

The idiopathic hypereosinophilic syndrome is defined as persistent eosinophilia of unknown origin. It is believed to be a reaction to an unidentified antigen or a inability of the organism to control its eosinophil production. The resultant eosinophilia is a systemic disorder that can be fatal, made manifest through the clinical signs of the affected organs. Eosinophilic invasion of tissues, associated with cytokine release and chemical mediators, determine organ damage and disfunction. Any organ can be affected, thus creating a puzzling clinical presentation. It commonly first affects the gastrointestinal tract, liver, spleen, bone marrow, lungs, and lymph nodes. Less frequently, it involves the skin, kidneys, heart, thyroid, adrenal glands and pancreas. It is believed that the Rottweiler is one of the breeds predisposed to this syndrome, alongside the German Shepherd, Siberian Husky, Alaskan Malamute and Cavalier King Charles Spaniel. We present the case of a Rottweiler with this rare disease and the steps taken to reach this uncommon diagnosis.

Keywords: *hypereosinophilic syndrome, Rottweiler, dog*

Introduction

Eosinophils are polymorphonuclear leukocytes that can be distinguished morphologically once specific secondary granules develop at the progranulocyte stage are nowadays considered pleotrophic multifunctional cells that serve complex physiologic roles (Weiss DJ and Wardrop KJ, 2010). Eosinophils develop in bone marrow and to a lesser extent in thymus, spleen, lung and lymph nodes, depending on the species, and their regulation depends on type 2 helper T (TH2) cells, which secrete IL-5 and IL-13. This includes increased production by bone marrow, mediated by IL-5 and recruitment to tissues by eotaxins, regulated by IL-13 (Weiss DJ and Wardrop KJ, 2010).

Eosinophils differentiate and mature in bone marrow over 2-6 days, depending on the species and comprise less than 10% of bone marrow nucleated cells (Weiss DJ and Wardrop KJ, 2010). The half-life of eosinophils in circulation in healthy individuals is around 1 hour in the dog. Eosinophils migrate into tissues (in particular the gastrointestinal tract and lungs), where they last for about 2 days unless anti-apoptotic factors, such as IL-5, prolong their survival for up to 2 weeks cells (Weiss DJ and Wardrop KJ, 2010; Meler E et al, 2010). Under pathologic conditions, it is possible for eosinophils to re-enter circulation (Dale DC et al, 1976). Activated eosinophils change in morphology, cell surface characteristics and functional activities (Dvorak et al, 1997). These changes usually appear after eosinophils leave circulation, but they may be found in the blood of patients with allergic disease and hypereosinophilic syndrome (Weiss DJ and Wardrop KJ, 2010).

Eosinophilia, defined as >1,500 eosinophils/μL of blood, is a frequent occurrence in dogs (Weiss DJ and Wardrop KJ, 2010). Eosinophilia occurs through inflammation and the elaboration of eosinophilopoietic factors (mainly IL-5) by T cells activated by parasite antigens or allergens (Herndon FJ and Kayes SG, 1992).

Both endoparasites and ectoparasites cause eosinophilia (Weiss DJ and Wardrop KJ, 2010). Chronic eosinophilia is associated with inflammation of mast cell-rich organs – skin, lung, GI tract and uterus, in all species, as well as with eosinophilic myositis, eosinophilic panosteitis and eosinophilic gastroenteritis in dogs (Mansfield C, 2008; Weiss DJ and Wardrop KJ, 2010). Paraneoplastic eosinophilia is caused by a variety of tumors, such as lymphoma, mast cell tumor and solid tumors, in which IL-5 and other cytokines are elaborated (Fernández-Aceñero MJ et al, 2000; Marchetti V et al, 2005).

Rarely, eosinophilia is reported after administration of certain drugs in the dog and has been associated with tetracycline and recombinant IL-2 administration (Weiss DJ and Wardrop KJ, 2010). Other causes of eosinophilia are presented in table 1. Chronic eosinophilic leukemia, a rare disease, must be differentiated from hypereosinophilic syndrome, where mild to moderate blood eosinophilia is accompanied by nonspecific tissue infiltration by eosinophils (Latimer et al, 2011) and the diagnosis depends on ruling out other causes and measuring serum IgE levels (Weiss DJ and Wardrop KJ, 2010).

Causes of Eosinophilia in Dogs and Cats

Hormonal Hypoadrenocorticism Oestrus in some bitches	**Infection** *Bacterial* *Fungal*, e.g. □Aspergillosis □Cryptococcosis *Parasites*, e.g. Aelurostrongylus abstrusus Ancylostoma spp. Angiostrongylus vasorum Capillaria aerophila Dirofilaria immitis
Immune mediated *Allergies* Atopy Feline asthma Flea allergy Food allergies Canine panosteitis Eosinophilic broncho-pneumopathy (dog) Eosinophilic gastroenteritis Eosinophilic granuloma complex Eosinophilic myositis Feline hypereosinophilic syndrome Pemphigus foliaceus	**Neoplastic** Eosinophilic leukaemia *Tumour-associated eosinophilia* □Fibrosarcoma □Myeloproliferative disease □Lymphoma □Mast cell tumour □Mucinous carcinomas □Transitional cell carcinoma

Note. Reprinted from Differential Diagnosis in Small Animal Medicine, Second Edition (p. 360-361), by A. Gough, K. Murphy, 2015, Pondicherry, India: SPi Publisher Services, Copyright 2015 by John Wiley & Sons, Ltd, Wiley-Blackwell

Table 1. Causes of Eosinophilia

Idiopathic hypereosinophilic syndrome (IHES) is described as a persistent eosinophilia of unknown origin and an increased survival of eosinophils in circulation, eosinophilic tissue infiltrates and consecutive organ dysfunction (Weiss DJ and Wardrop KJ, 2010). In humans, idiopathic hypereosinophilic syndrome is defined by sustained (over 6 months) peripheral eosinophilia of >1,500 cells/μL with no discernible cause and multiple organ involvement - gastrointestinal tract, liver, spleen, bone marrow, lungs, lymph nodes, skin, kidneys, heart, thyroid, adrenal glands and pancreas (Lilliehöök I et al., 2000; Weller PF and Bubley GJ, 1994; Sykes et al, 2001). The difference between idiopathic hypereosinophilic syndrome and eosinophilic leukemia is difficult to establish and in some cases differentiation may not be possible (Sykes et al, 2001). One difference to be considered is that the maturation of eosinophils is regular in hypereosinophilic syndrome, while marked eosinophilic left shifts and bone marrow, blood and organ infiltrates are more likely in eosinophilic leukemia (Harvey JW, 2001). One mechanism suggested for eosinophilia is the clonal expansion of T cells generating eosinophilopoietic factors (Weller PF and Bubley GJ, 1994), and this increase in IL-5 levels can prevail over the apoptotic effects of corticosteroids; eosinophilia is sometimes observed in animals with hypoadrenocorticism due to decreased or absent cortisol (Weiss DJ and Wardrop KJ, 2010).

Of all dog breeds, Rottweilers are the most predisposed to eosinophilic diseases, having increased eosinophilic values of no identifiable cause (parasitic, allergic or neoplastic) or age or

sex predisposition (Mansfield C, 2008). There seems to be a heritable component to eosinophilia (Mansfield C, 2008). Rottweilers are also the most frequently affected by hypereosinophilic syndrome. Sykes et al (2001) diagnosed 3 dogs with IHES on the basis of a lack of immature eosinophils and karyotype abnormalities (as opposed to eosinophilic leukemia), increased mean serum IgE concentration and the absence of an apparent cause. The absence of clonal karyotype abnormalities does not rule out underlying neoplasia; in human medicine, some patients with eosinophilic leukemia manifest cytogenetic abnormalities later on (Sykes et al, 2001; Rothenberg ME, 1998), therefore any patient diagnosed with IHES should be regularly monitored.

The treatment of IHES in humans is based on glucocorticoids, which suppress cytokine gene transcription and inhibit cytokine-dependent eosinophil survival (Sykes et al, 2001). Patients resistant to glucocorticoids can be treated with hydroxyurea, vincristine, interferon or cyclosporine (Perkins MC, Watson AD, 2001; Lilliehöök I, Tvedten H, 2003). In veterinary medicine, the disease is more frequently described in cats than in dogs, but due to the small number of cases reported, the prognosis and best treatment options are not yet established (Ferian PE et al, 2017). Although it can be fatal in animals presenting with severe clinical symptoms, spontaneous remission is possible (Ferian PE et al, 2017; James FE and Mansfield CS, 2009). In human medicine, the main cause of death is eosinophilic cardiomyopathy due to infiltration and subsequent myocardial necrosis, mural thrombus formation and eventually subendocardial and endocardial fibrosis, culminating with congestive heart failure due to restrictive cardiomyopathy (Perkins MC, Watson AD, 2001)..

Materials and methods

Complete blood counts were performed at Synevovet Laboratory and in-house using a Mindray BC-2800 Vet automatic hematology analyzer. Blood biochemistry was performed in-house using a Rayto RT-1904C semiautomatic chemistry analyzer and at Synevovet. The cytological examinations were performed by Dr. Teodoru Soare. The cardiac examination was performed by dr. Florin Leca at Doctor's Vet Univers. The radiologic examinations were performed at 4VET Radiology Center and interpreted by dr. Florin Grosu. The ultrasonographic examinations were performed with a portable color Doppler Sonoscape S2 system by dr. Otilia Cristea.

Case presentation

Becko, a 4 year old fully intact Rottweiler, was presented to the vet for malaise and a loss of appetite. The clinical examination revealed fever (40ºC), tachycardia, tachypnea, generalised lymph node reactivity and a distended abdomen. Becko had always been correctly vaccinated and given internal and external parasite preventives.

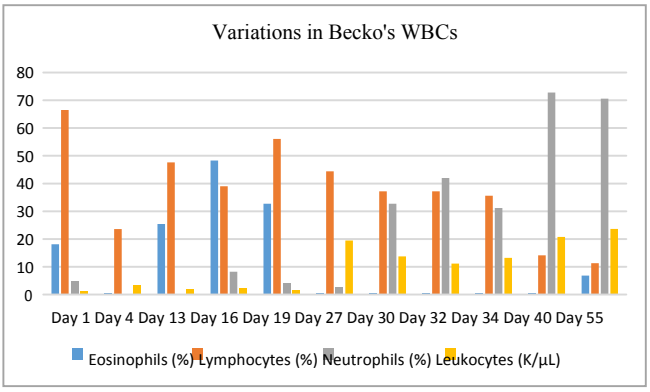

Figure 1. Significant WBC changes over time

An in-house complete blood count (CBC) revealed intense neutropenia and eosinophilia, monocytopenia, lymphocytosis, mild non-regenerative anemia, decreased hematocrit and hemoglobin. The biochemisty revealed decreased albumin, increased total protein (TP) and mild hypocalcemia. Troponin I was 0.01 ng/mL (reference <0.08) showing no signs of myocardial injury. Blood cytology identified no signs of parasites/bacteria or hyperplastic/neoplastic cells.

Ultrasonography of the abdomen revealed an enlarged but homogenous spleen and reactive abdominal lymph nodes. The dog was started on intravenous ceftriaxone and subcutaneous dexamethasone alongside supportive treatment.

The next day, the dog's state deteriorated and a procalcitonin titer of 2.5 ng/mL was obtained (reference value <0.5 ng/mL), supportive of a systemic infection and a high risk of sepsis. The dog presented with fever, lethargy, tachypnea and tachycardia. Becko responded to the treatment (his temperature dropped to 39ºC and he started to eat) after 4 days and the iv antibiotic was replaced with oral cephalexin. Dexamethasone was administered daily for 2 weeks beginning on the first day. Compensatory tachycardia and tachypnea continued despite the absence of fever due to the ensued anemia (figure 2).

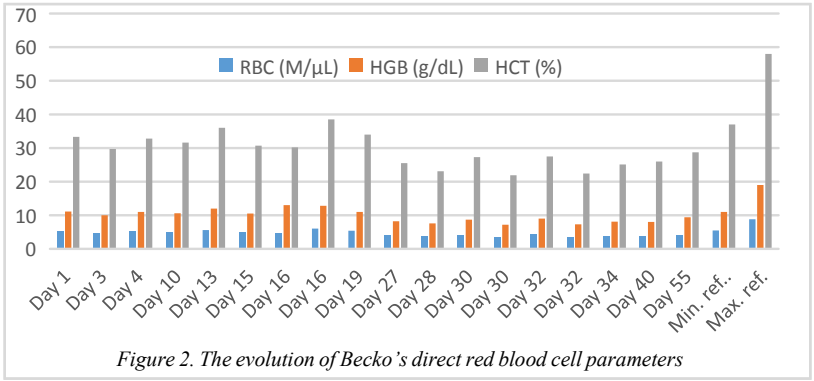

Figure 2. The evolution of Becko's direct red blood cell parameters

The cytologic examination of a popliteal lymph node aspirate revealed a heterogenous population of small, medium and large lymphocytes and plasmocytes and no evidence of neoplastic cells in the examined slides, consistent with a reactive lymph node. The superficial lymph nodes continued to be clinically enlarged and reactive for approximately 5-7 days.

On day 8, after 4 days on oral antibiotics, the fever returned (41ºC). Becko was once again not eating and lethargic and his procalcitonin level was 2 ng/mL. He restarted iv ceftriaxone and was administered one dose of Theranekron, a homeopathic remedy prepared from the spider Tarantula cubensis, for its antiinflammatory properties. Blood biochemistry revealed low albumin, increased total protein, creatine kinase and alkaline phosphatase. The CBC revealed a mild regenerative anemia (RBC 4.9 M/µL, HGB 10.6 g/dL, HCT 31.6%) which gradually worsened over the next weeks (figure 2). Supportive treatment was continued throughout the period the dog was not eating on his own.

As the fever continued, Becko was reffered for thoracic radiographs and a cadiac examination to exclude the possibility of bacterial endocarditis. The X-rays revealed a bronchial pattern indicative of a infectious or inflammatory disease and a physiological vertebral heart score. At the time of examination, the cardiologist identified a heart rate of 137 bpm, a capillary refill time of 2 seconds, normal mucous membrane color, no abnormalities of the peripheral pulse and normal breath sounds. With an increased PQ interval, Becko was diagnosed with a first degree atrioventricular block and scheduled for quarterly examinations. Ecocardiography did not reveal any changes of the heart or its function.

Two weeks after the initial episode, Becko was put on intravenous levofloxacin and meropenem, as his fever stopped responding to ceftriaxone. Despite being treated with dexamethasone, Becko had a marked eosinophilia and dexamethasone was replaced with prednisolone. The eosinophilia was suspicious, as Becko's mother and 3 other male brothers had a history of an unexplicably increased eosinophil counts. We step by step investigated and ruled out the causes of eosinophilia (see table 1). The serum IgE level was determined twice with two week interval and was found to be normal, indicating that an allergic process is unlikely to be present. Toxoplasma gondii IgG and IgM (Synevovet) titers were <1:100 and considered negative. Repeated SNAP 4Dx Plus tests (IDEXX Laboratories, Inc.) were negative to all six vector-borne diseases. Coproparasitologic examinations were performed on three consecutive days and at 7, 10 and 14 days using feces from each stool, from three different areas and from each fragment whose colour or texture were modified and they were all negative. Due to the continuous anemia, we tested a blood sample for the presence of Haemobartonella antigen but the test was negative. The dog had been eating the same food for over a year, there was no sign of gastroenteritis, any difficulty walking or any skin lesions. Eosinophilia was intermittently observed despite no evidence of allergic disease or other causes and treatment with corticosteroids (figure 3). Ultrasonographically, there was moderate hepato- and splenomegaly with a diffuse variation in echogenicity and slightly irregular margins. The abdominal lymph nodes were no longer visibly enlarged.

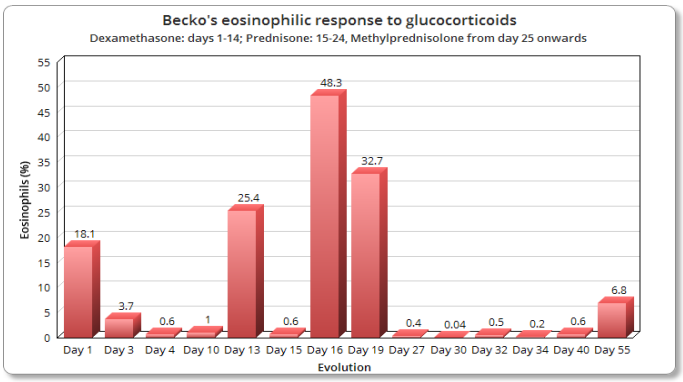

Figure 3. Eosinophilia in response to glucocorticoids. The blood sample on day 1 was obtained before treating with dexamethasone.

The antibiotic was continued for 10 days and Becko showed signs of improvement on the second day of this regimen. His evolution was favourable and after a few days he was given oral methylprednisolone at 0.5 mg/kg, dose which was gradually increased to 2 mg/kg.

Taking into account the history, paraclinical evidence and ultrasonographic changes, and considering Becko's familial history, we suspected a case of idiopathic hypereosinophilic syndrome, overrepresented in Rottweilers. Due to the case's evolution, the previous septic and inflammatory processes, the unexplicable fever and anemia and the continuous CBC variations, a bone marrow aspirate was submitted for a cytologic examination. The slide revealed a normal myeloid:erythroid ratio of 2:1 and the presence of all precursor cells for the erythroid, lymphoid and myeloid lineages.

The confirmation came when the result indicated that over 28% of the myeloid cells were eosinophil precursors (in-house reference: <5%) and no signs of neoplasia in the examined cells. At the same time, concomitant blood cytology did not reveal eosinophilic precursors in the blood stream, which excluded, for now, the possibility of a eosinophilic leukemia. Becko's mother and brothers had had episodes of unexplicable eosinophilia on yearly routine CBCs. In particular, the

mother, which had been tested before each mating, recorded values which were never less than 5% eosinophils, manifesting a seasonal pattern with higher values in spring and autumn, when she would reach > 10% eosinophils.

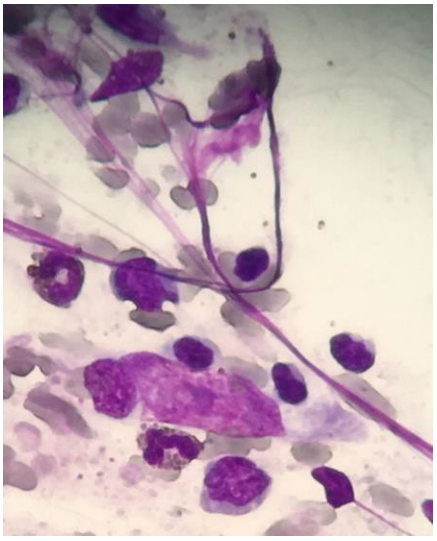

Figure 2. Photomicrograph of bone marrow fine needle aspiration, MGG, 1000x, showing pronounced eosinophilic hyperplasia, undifferentiated myeloid precursors with an eosinophilic differentiation and normal morphology.
Source: prepared by Dr. T. Soare

Unfortunately, Becko's state gradually worsened, manifesting progressive generalised diffuse amyotrophy, including atrophy of the respiratory muscles and finally cachexia. The most significant change in blood biochemistry was hypoalbuminemia. Ultrasonography revealed large quantities of anechoic fluid in both body cavities and diffuse infiltrates of the abdominal organs, lungs and heart.The pleural and peritoneal fluids were examined cytologically and revealed a large number of eosinophils. Becko died of a cardiopulmonary arrest and the owner declined necropsy.

Conclusions

The first clinical sign Becko manifested was the fever of unidentified origin.

The helpful diagnostic clues were the persistent recurrent eosinophilia despite glucocorticoid therapy and the familial history (the mother and brothers always registered over 5% eosinophils and occasionally over 10%, in particularly the male brothers).

These, coupled with the unexplicable pyrexia, led to a suspicion of a genetic disease. Not every dog presenting with eosinophilia has idiopathic hypereosinophilic syndrome and it is essential to rule out the causes of increased eosinophil counts.

If all investigations point to an idiopathic process, the dogs with persistent eosinophilia might benefit from early glucocorticoid therapy, before the onset of clinical disease, and should be subsequently subjected to regular clinical and paraclinical examinations.

We observed that the dog responded better to prednisolone and methylprednisolone than to dexamethasone, as suggested in the literature. In severe cases, treatment seems to be illusive, therefore we recommend that eosinophilia in susceptible breeds, in particular the Rottweiler, be investigated thoroughly and effort be made to examine the other littermates and parents.

Bibliografie

1. Aroch I et al (2001) Disseminated eosinophilic disease resembling idiopathic hypereosinophilic syndrome in a dog. Vet Rec 149(13):386-389
2. Clercx C, Peeters D, Snaps F, Hansen P, McEntee K, Detilleux J, Henroteaux M, Day MJ (2000) Eosinophilic bronchopneumopathy in dogs, J Vet Intern Med 14(3):282-291
3. Clercx C, Peeters D (2007) Canine Eosinophilic Bronchopneumopathy, Vet Clin Small Anim 37 917–935
4. Cowgill E, Neel J (2003) Pleural fluid from a dog with marked eosinophilia. Vet Clin Pathol 32(3):147-149
5. Dale DC, Hubert RT, Fauci AS (1976) Eosinophil kinetics in the hypereosinophilic syndrome, J Lab Clin Med 87: 487-495.
6. Drouot S et al (2007) Acute idiopathic hypereosinophilic syndrome in a rottweiler. Schweiz Arch Tierheilkd 149(11):511-516
7. Dvorak AM, Ackerman SJ, Weller PF (1991) Subcellular morphology and biochemistry of eosinophils, Blood Cell Biochemistry, Vol 2, New York, p. 234-37
8. Ettinger SJ, Feldman EC (2011) Textbook of Veterinary Internal Medicine 7th revised edition, Ch. 193, Elsevier Health Sciences, London, United Kingdom
9. Ferian PE; Bach EC; Zanine Salbego F; Zorzi Madaloz L; Volpato J; Rinaldi Muller T; Carneiro RA (2017) Idiopathic hypereosinophilic syndrome in a rottweiler: a case report, Semina: Ciências Agrárias, vol. 38, núm. 1, enero-febrero, 2017, pp. 311-316, Universidade Estadual de Londrina, Londrina, Brasil
10. Fernández-Aceñero MJ, Galindo-Gallego M, Sanz J et al (2000) Prognostic influence of tumor-associated eosinophilic infiltrate in colorectal carcinoma, Cancer 88: 1544-1548
11. German AJ et al (2002) Eosinophilic diseases in two Cavalier King Charles spaniels. J Small Anim Pract 43(12):533-538
12. Gough A, Murphy K (2015) Differential Diagnosis in Small Animal Medicine, Second Edition Pondicherry, India, John Wiley & Sons, Ltd, Wiley-Blackwell
13. Harvey JW (ed.) (2001) Atlas of Veterinary Hematology, Saunders Elsevier, USA
14. Herndon FJ , Kayes SG (1992) Depletion of eosinophils by anti-IL-5 monoclonal antibody treatment of mice infected with Trichinella spiralis does not alter parasite burden or immunologic resistance to reinfection, J Immunol 149: 3642-3647
15. James F. E.; Mansfield C. S. (2009) Clinical remission of idiopathic hypereosinophilic syndrome in a Rottweiler, Australian Veterinary Journal, Victoria, v. 87, n. 8, p.330-333
16. Latimer KS, Mahaffey EA, Prasse KW (eds.) (2011) Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology, 5th Ed., Wiley-Blackwell
17. Lilliehöök I, Gunnarsson L, Zakrisson G, Tvedten H (2000) Diseases associated with pronounced eosinophilia: a study of 105 dogs in Sweden, J Small Anim Pract. 41(6):248-53
18. Lilliehöök I, Tvedten H (2003) Investigation of hypereosinophilia and potential treatments. Vet Clin North Am Small Anim Pract 33(6):1359-1378
19. Mansfield C (2008) Eosinophilic Diseases of Dogs, World Small Animal Veterinary Association World Congress Proceedings
20. Marchetti V et al (2005) Paraneoplastic hypereosinophilia in a dog with intestinal T-cell lymphoma. Vet Clin Pathol 34(3):259-263
21. Meler E et al (2010) Diffuse cylindrical bronchiectasis due to eosinophilic bronchopneumopathy in a dog. Can Vet J 51(7):753-756
22. Perkins MC, Watson AD (2001) Successful treatment of hypereosinophilic syndrome in a dog. Aust Vet J 79(10):686-689
23. Rothenberg ME (1998) Eosinophilia, N Engl J Med 338:1592–1600
24. Sykes JE et al (2001) Idiopathic hypereosinophilic syndrome in 3 Rottweilers, J Vet Intern Med 15(2):162-166
25. Weiss DJ, Wardrop KJ (eds.) (2010) Schalm's Veterinary Hematology, Chapter 43, Eosinophils and their disorders by Young KM and Meadows RL, Blackwell Publishing, SUA
26. Weller PF, Bubley GJ (1994) The idiopathic hypereosinophilic syndrome, Blood 83:2759–2779